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Effects of Extracts of *Coriolus versicolor* (I'm-Yunity™) on Cell-Cycle Progression and Expression of Interleukins-1β, -6, and -8 in Promyelocytic HL-60 Leukemic Cells and Mitogenically Stimulated and Nonstimulated Human Lymphocytes

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ABSTRACT

Objective: The goal of this *in vitro* study was to test the cytostatic and cytotoxic activities of extracts derived from the polysaccharopeptide (PSP), I'm-Yunity,™ (Integrated Chinese Medicine Holdings Ltd., Kowloon, Hong Kong) prepared from strain Cov-1 of the mushroom *Coriolus versicolor*.

Design: Different volumes of 70% ethanol and water extracts of I'm-Yunity were incubated with cultures of human promyelocytic leukemic HL-60 cells, and compared to nontreated control cells. At various times after treatment, cells were harvested and analyzed with respect to: (1) proliferation and cell cycle phase distribution, (2) induction of apoptosis, and (3) changes in expression of the immunomodulating cytokines interleukin (IL)-1β, IL-6, and IL-8. To test whether extracts also affected normal cells, similar experiments were also performed using isolated peripheral blood lymphocytes from healthy volunteers, with and without stimulation by the mitogen phytohemagglutinin (PHA). The ability of extracts to affect the secretion of IL-1β, IL-6, and IL-8 were assessed by enzyme-linked immunosorbent assay.

Results: HL-60 cells incubated with various amounts (1, 3, 5, 7.5, and 10 μl/mL) of the extracts for 1–3 days showed dose-dependent, time-dependent growth suppression and decrease in cell viability. Flow cytometric analysis revealed partial cell arrest in the G_1 phase at less than 5 μL/mL and induction of apoptosis at 10 μL/mL or more of ethanol and water extracts, with the latter exhibiting more pronounced inhibition than the former. Experiments performed with lymphocytes demonstrated that extracts of I'm-Yunity alone were without effect; moreover, they also did not affect the lymphocyte response to PHA. Water extract of I'm-Yunity also significantly increased IL-1β and IL-6 while substantially lowering IL-8.

Conclusions: I'm-Yunity acts selectively in HL-60 leukemic cells, resulting in cell cycle restriction through the G_1/S checkpoint and the induction of apoptosis.

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INTRODUCTION

Epidemiologic studies over the past several decades have conclusively demonstrated that there are significant regional as well as racial/ethnic differences with respect to age-adjusted incidence and mortality rates for a number of cancers (Armstrong and Doll, 1975; Doll and Peto, 1981; Greenlee et al., 2001; Koo and Ho, 1990; Ziegler et al., 1993). Genetic and epigenetic factors are believed to be involved in the observed tumor incidence and mortality variations; the weight of evidence, however, points to nongenetic factors as having a more critical influence in the observed outcome. Only a very minor proportion of all cancers, perhaps no greater than 5%, is linked to genetic considerations, the majority is associated with environmental conditions acting in concert with individual susceptibility factors (Lynch et al., 1997; Perera, 1996; Perera and Weinstein, 2000). Identification and characterization of these factors, both environmental and lifestyle, are intensively researched in many laboratories.

Ample data from *in vitro*, animal, and migrant studies point to the involvement of plant-derived agents in conferring protection against tumorigenesis (Kolonel, 1988; Riboli, 2001; Ziegler et al., 1996). Consumption of vegetables, fruits, and whole grains is strongly and significantly associated with a reduction in incidence and mortality in a number of malignancies (Hill, 1997; Miller, 1990; Sinha and Caporaso, 1999). However, whether culture-specific cooking practices and dietary preferences—including ones that emphasize use of high heat and multiple herbs or herb-derived extracts, both to preserve food and enhance its flavor—influence cancer initiation, progression, and establishment have not been extensively investigated. We hypothesize that these aspects of nutrition and diet could be just as important as the consumption of vegetables and fruits for cancer prevention. Indeed, recent data show that specific herbal and dietary ingredients, for example, ursolic acid in rosemary (Lee et al., 1999; Subbaramaiah et al., 2000), licochalcone in licorice (Rafi et al., 2000), and resveratrol in grapes (Hsieh and Wu, 1999; Jang et al., 1997; Mollerup et al., 2001), are effective chemopreventive agents in model systems.

Various mushrooms are consumed as culinary delicacies. In addition, they also display antibacterial, antifungal, antiviral, and antitumor activities (Borchers et al., 1999; Kidd, 2000; Ng, 1998; Wasser and Weis, 1999) and the ability to augment immune functions (Borchers et al., 1999; Kidd, 2000). *Coriolus versicolor*, known in China as Yun-Zhi, is a mushroom belonging to species of the *Basidiomycetes* class of fungi. The medicinal value of these fungi was recorded thousands of years ago in the *Shen Non Compendium Medica* (Wasser and Weis, 1999; Yang, 1997). Recent studies in China and elsewhere have confirmed the fungi's therapeutic potentials (Kidd, 2000; Yang, 1997). The bioactive agent in this mushroom is believed to be a polysaccharopeptide (PSP) that has an efficacy that probably derives from its ability to interact with pathogens, hormones and toxins, and perhaps participation in cell–cell communication (Kidd, 2000; Yang, 1997).

The diverse biologic roles that PSP appear to play offers the appealing possibility that it may have preventive or complementary pharmaceutical applications against a number of diseases with long latencies and defined stages of progression, while exerting minimal effects on normal cells. Here we test this hypothesis by studying the effects of the PSP I'm-Yunity™ (Integrated Chinese Medicine Holdings Ltd., Kowloon, Hong Kong), prepared from the cultivated Cov-1 strain of *Coriolus versicolor*, in human leukemia HL-60 cells and in control and phytohemagglutinin (PHA)-stimulated peripheral lymphocytes. Parameters measured included: (1) proliferation and cell cycle progression; (2) induction of apoptosis; and (3) expression of the cytokines interleukin (IL)-1β, IL-6, and IL-8. Results of our studies show that water and 70% ethanol extracts of I'm-Yunity elicited a dose-dependent and time-dependent suppression of HL-60 cell growth, accompanied by a partial arrest in the G_1 phase of the cell cycle. In addition to being more active than the ethanol extract, the water extract also significantly increased secreted IL-1β and IL-6 while substantially lowered IL-8, in HL-60 cells. Few effects were observed when extracts

were incubated with control and PHA-stimulated human lymphocytes.

MATERIALS AND METHODS

Peripheral blood lymphoytes

Blood was obtained from healthy human volunteers by venipuncture, and peripheral blood lymphocytes (PBL) were isolated by density gradient centrifugation as described (Juan et al., 1998). The PBL were washed twice with Hanks' buffered salt solution (HBSS) and resuspended at a density of 10^6 cells per milliliter in RPMI-1640 supplemented with 10% fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L L-glutamine. Isolated PBL were treated with 10 μg/mL PHA to stimulate mitogenesis. Media, supplements, and antibiotics used to culture PBL were obtained from Life Technologies (Grand Island, NY). PHA was purchased from Sigma Chemical Co. (St. Louis, MO).

Preparation of ethanolic and water extracts from I'm-Yunity

I'm-Yunity was provided by Integrated Chinese Medicine Holdings Ltd. and stored at 4°C. According to the manufacturer, I'm-Yunity was obtained from deep-layer cultivated mycelia of *Coriolus versicolor* strain Cov-1, as follows. The mycelia were extracted with water at a proprietary temperature for 4–5 hours, followed by differential precipitation with ethanol. Further processing of specific ethanol fractions led to isolation of I'm-Yunity, with molecular weights greater than 40 kd and an average polysaccharide:peptide ratio of 2:1. Figure 1 shows a typical high-performance liquid chromatography (HPLC) profile of the water extract of I'm-Yunity used in the present investigation. To test its biologic activity, the content of each capsule (containing 340 mg of powder) was mixed with 1 mL of 70% ethanol or water. The suspension was stirred with intermittent mixing at 150 rpm for 60 minutes at room temperature. The insoluble material was removed by centrifugation in a microcentrifuge and the soluble supernatant was sterilized using a 0.22-μm filter. Before use, the stock extract kept at 4°C was diluted with tissue culture media to give the final concentrations indicated in various experiments. Two lots were prepared and tested.

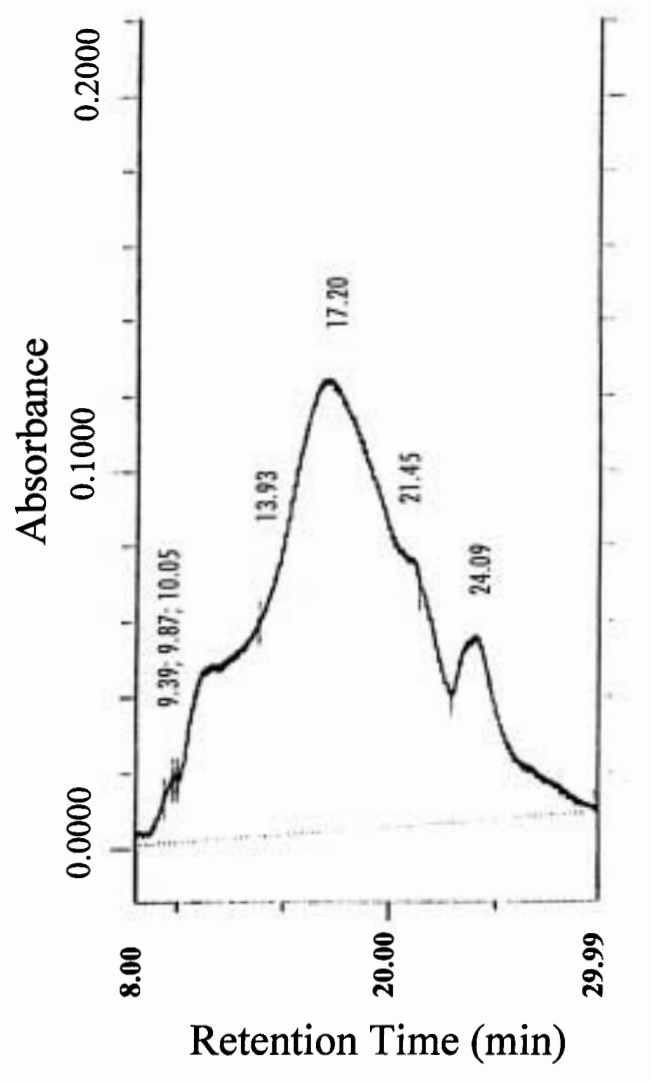


FIG. 1. Analysis of I'm-Yunity™ (Integrated Chinese Medicine Holdings Ltd., Kowloon, Hong Kong) by high-performance liquid chromatography (HPLC). Water extracts of I'm-Yunity prepared as described in Materials and Methods were subjected to high-performance liquid chromatography (HPLC) fractionation using TSK G4000SW. The column was eluted with 0.005 mol sodium acetate buffer, pH 6.5 at a flow rate of 0.5 mL/min. The elution profile was monitored by refractive index.

Cell culture and treatment of HL-60 cells with mushroom extracts

Human promyelocytic HL-60 leukemia cells were obtained from the American Type Cul-

ture Collection (ATCC, Rockville, MD) and cultured as described previously (DiPietrantonio et al., 1998, 2000). In a typical experiment, 5 mL of cells at a density of 1×10^5 cells per milliliter were seeded in T25 flasks. Different amounts of ethanol or water extracts were added into the culture media. Where appropriate, control cells were incubated with the same volumes of 70% ethanol as used in treated cells. At the indicated times, cell count was performed using a hemocytometer and cell viability was determined by trypan blue exclusion. Cells were harvested, washed twice with phosphate-buffered saline (PBS), and pellets were stored at −80°C for further analysis.

Effects of I'm-Yunity on cell cycle progression

Cell cycle phase distribution was evaluated using flow cytometry. After a 3-day treatment of HL-60 cells or PBL with different amounts of the extracts (1, 3, 5, 7.5, and 10 μL/mL), cells were washed with PBS and stained with 1.0 μg/mL DAPI containing 100 mmol/L NaCl, 2 mmol/L $MgCl_2$, and 0.1% Triton X-100 (Sigma) at pH 6.8, as previously described (Darzynkiewicz and Bedner, 2000; Hsieh et al., 1999). The blue (DNA-specific) DAPI fluorescence was excited with an ultraviolet light–emitting laser (Ni-Cad) for DAPI excitation, and collected with appropriate filters in an ICP-22 (Ortho Diagnostic, Westwood, MA) flow cytometer. MultiCycle software (Phoenix Flow Systems, San Diego, CA) was used to deconvolute the cellular DNA content frequency histograms to obtain the percentage of cells in the different phases (G_1, S and G_2/M) of the cell cycle. Flow cytometry was also used to demonstrate cells undergoing apoptosis, evidenced by the appearance of the sub-G_1 peak (DiPietrantonio et al., 1996).

Enzyme-linked immunosorbent assay

IL-1β, IL-6, and IL-8 were determined using solid phase enzyme-linked immunosorbent assay (ELISA). The quantitative sandwich enzyme immunoassay utilizes a microplate-based protocol designed to quantitatively measure various ILs in cell culture supernatants. Specifically, microplates precoated with different monoclonal antibodies permit quantitative and selective binding of a specific IL present in the tissue culture supernatants and in the standard IL solutions provided by the manufacturer. After binding, unbound substances were removed by washing. IL-specific enzyme-linked polyclonal antibodies were added to the wells, followed by additional washing to remove the unbound antibody-enzyme reagent. The last step involved the addition of substrates that when reacted with the antibody-conjugated enzyme, yielded colors whose measured intensity in a microplate reader was proportional to the amount of ILs bound in the initial step. For each IL tested, the assays were done in triplicate using varying amounts of culture supernatants. Accuracy of these assays was verified by comparison with curves generated using standard ILs.

RESULTS

Effects of ethanol versus water extracts of I'm-Yunity on growth of HL-60 cells

Bioactive ingredients selectively soluble in ethanol or water could coexist in mushroom extracts. Accordingly, powder contained in capsules was extracted with 70% ethanol or water, as described in Materials and Methods. Biologic activity of the extracted material was tested using the human promyelocytic HL-60 leukemic cells. This cell line, originally isolated from the peripheral blood of a patient with acute promyelocytic leukemia, has been a model system for screening antiproliferative agents from a variety of sources, and for identifying candidate compounds capable of inducing cell differentiation and apoptosis (DiPietrantoni et al., 1996, 1998, 2000). Varying amounts of 70% ethanol and water extracts were incubated with HL-60 cells and proliferation was measured at different times. Figure 2A shows the change in HL-60 cell number after a 3-day incubation with 0, 1, 3, 5, 7.5, and 10 μL/mL of extracts. Both 70% ethanolic and water extract resulted in dose-dependent growth suppression, with the latter eliciting a more pronounced reduction in cell growth. We estimated that IC_{50} of the water extract was 3–5 times lower than that of the 70% ethanol extract because 1 μL/mL of the former gave a

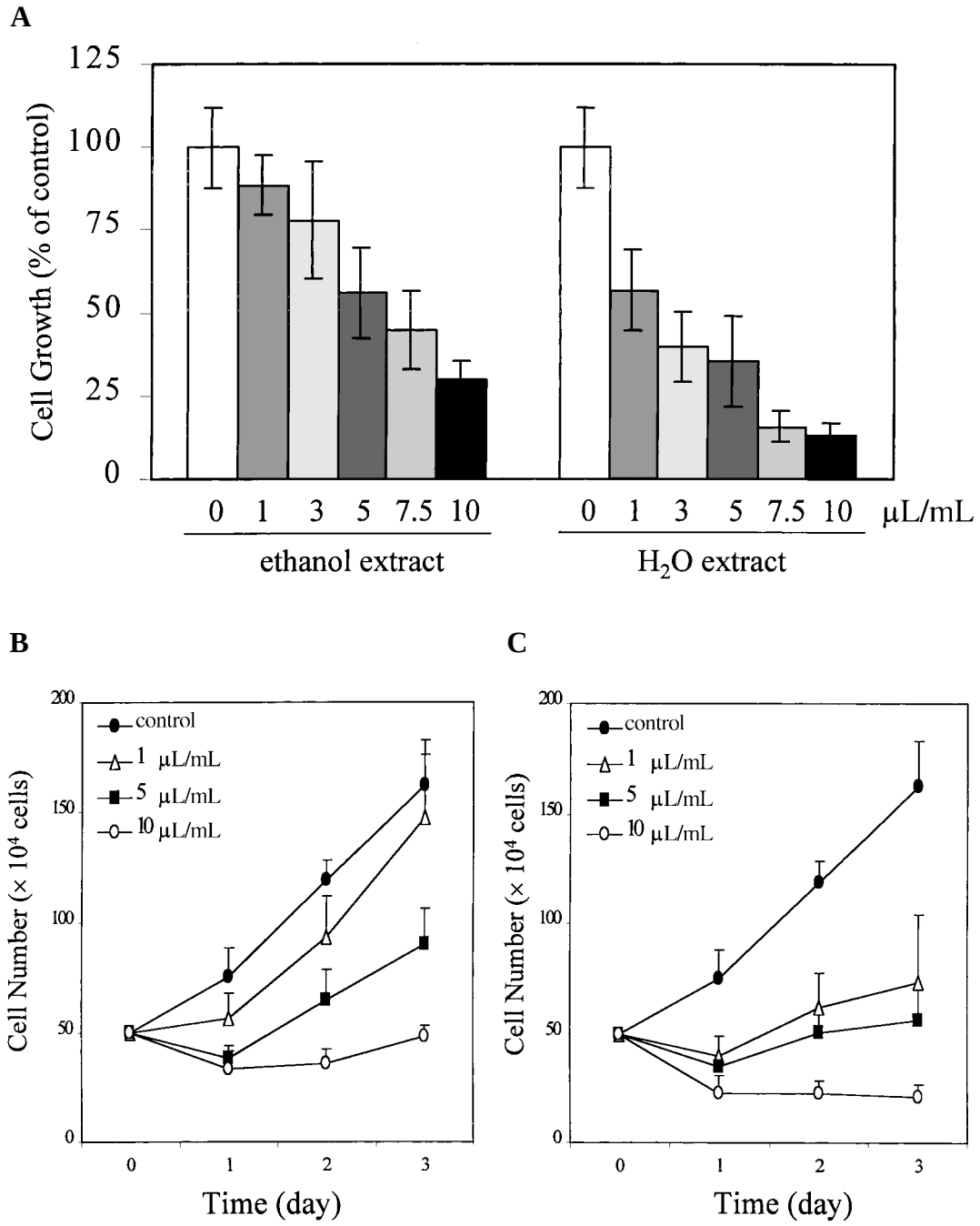


FIG. 2. Cell cycle effects of different concentrations of 70% ethanol and water extracts of I'm-Yunity™ (Integrated Chinese Medicine Holdings Ltd., Kowloon, Hong Kong) in HL-60 cells. **A:** cells were treated for 72 hours with the indicated concentration of either extract, as described in Materials and Methods. Growth was monitored by counting cell number using a hemacytometer. Control cell number was set at 100%, and treated cell number was expressed as a percentage of controls. **B:** Time-dependent inhibition of HL-60 cells growth by 70% ethanol extract of I'm-Yunity. **C:** Time-dependent inhibition of HL-60 cells growth by water extracts of I'm-Yunity. Results represent the average of 2–3 experiments using two different lots of I'm-Yunity.

comparable suppression of growth as 5 μL / mL of the latter (Fig. 2A). Figures 2B and 2C depict time-dependant and dose-dependent suppression of cell growth by both extracts. With the 70% ethanol extract, the effect was primarily cytostatic (Fig. 2B). The water extract, however, displayed both cytostatic and cytotoxic attributes; at the highest concentration tested, the ab-

solute cell number at day 3 was substantially decreased compared to the number of cells seeded (Fig. 2C).

Effects of extracts on HL-60 cell cycle phase distribution and induction of apoptosis

Although the popular approach for tackling tumorigenesis since the 1960s has relied on use of cytotoxic agents, often in combination with compounds capable of inducing cell differentiation, another dimension in the management of tumor has surfaced in the last decade. The broad theme of this approach focuses on cell death and is based on the realization that tumor growth reflects equilibrium between proliferation and cell death rates. To gain insights on the cytostatic and cytotoxic attributes described above, we analyzed cell cycle distribution using flow cytometry. Incubation of HL-60 cells with either extracts resulted in a significant change in the cell cycle distribution, with cell accumulation in the G_1 and a concomitant decrease in proportion of cells in the S phase. For instance, there was a nearly twofold decrease in S phase (from 41.2% to 23.8%) by 10 μL / mL ethanol extract compared to controls. With water extract, the highest dose elicited an almost fourfold decrease, from 41.2% to 11.3%, paralleled by increases in G_1 and G_2M-phases of the cell cycle, as well as by substantial induction of apoptosis, which was identified as the cells with fractional DNA content (Darzynkiewicz and Bedner, 2000) (Table 1). These data agreed with results obtained by counting cell number and evaluating cell viability (Fig. 2).

Effects of extracts in isolated PBL

To test whether extracts may exhibit a similarly cytostatic / cytotoxic effects on normal cells, extracts were added to control and PHA-stimulated lymphocytes. Results presented in Figure 3 show that up to 5 μL / mL of water extract did not affect cell growth and cell cycle distribution in control and PHA-stimulated lymphocytes at 72 hours. Ethanolic extracts also were without effects (data not shown).

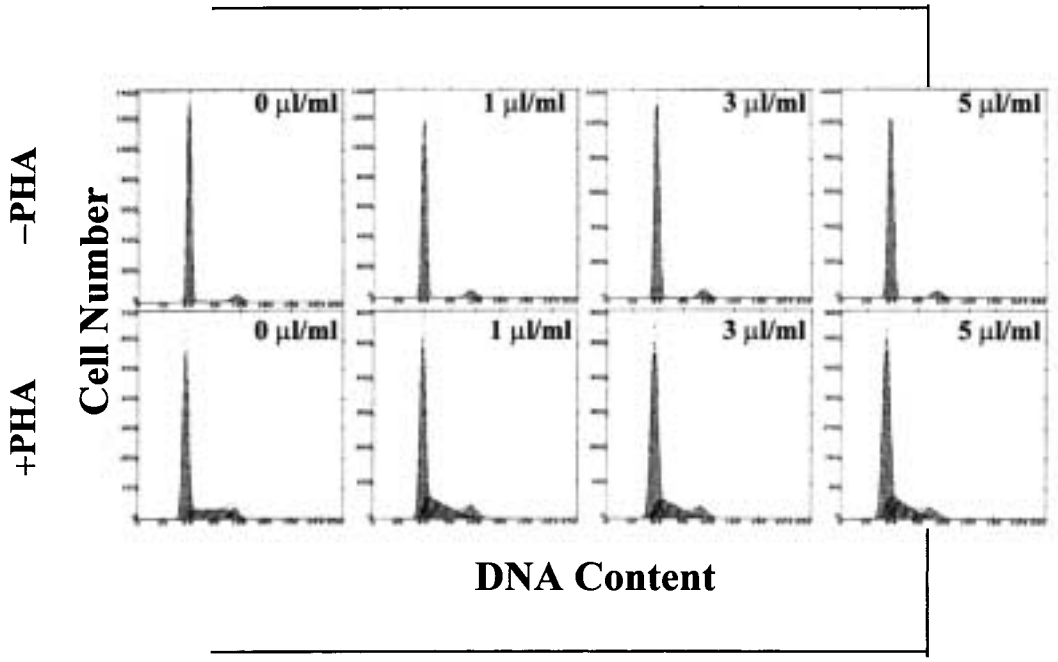
Effects of extracts on secretion of ILs

Because mushroom extracts have been reported to affect immune functions (Borchers et al., 1999; Kidd, 2000), we assessed changes in several ILs, using cell media derived from control and treated HL-60 cells. Results presented in Figure 4 show changes in IL-1β, IL-6, and IL-8. The first two ILs increased significantly, especially after 3 days of treatment with the higher concentration of both extracts, with water extract eliciting a more pronounced change (Fig. 4). IL-8 decreased in response to the addition of both extracts (Table 2).

TABLE 1. EFFECTS OF SEVENTY-TWO–HOUR TREATMENT OF HL-60 CELLS WITH VARIOUS CONCENTRATIONS OF ETHANOL OR AQUEOUS EXTRACTS OF I'M-YUNITY™ [a]

Extract	*Treatment (μL/mL)*	*Cell-cycle distribution*			
		G_1	*S*	*G_2/M*	*Apoptosis*
	Control	47.4 ± 0.1	41.2 ± 1.1	11.4 ± 1.0	
Ethanol (70%)	1.0	47.8 ± 1.6	42.3 ± 2.7	10.0 ± 1.0	
	3.0	50.1 ± 0.5	39.4 ± 0.4	10.6 ± 0.1	
	5.0	53.4 ± 2.0	37.5 ± 2.4	9.1 ± 4.4	
	7.5	59.9 ± 3.5	29.8 ± 4.5	10.4 ± 1.0	
	10.0	61.5 ± 1.6	23.8 ± 0.8	14.8 ± 2.3	4.2 ± 2.4
H_2O	1.0	53.9 ± 0.4	37.2 ± 0.6	9.0 ± 0.2	
	3.0	58.1 ± 1.4	33.7 ± 3.0	8.2 ± 1.6	
	5.0	65.3 ± 1.7	27.6 ± 1.3	7.1 ± 0.4	
	7.5	72.4 ± 1.5	10.7 ± 2.1	17.1 ± 0.5	11.4 ± 2.2
	10.0	73.8 ± 3.5	11.3 ± 7.1	14.9 ± 3.5	42.8 ± 6.3

[a]Integrated Chinese Medicine Holdings Ltd., Kowloon, Hong Kong.
H_2O, water.



PBL	Time (h)	I'm-Yunity™ (μl/ml)	Cell Cycle Distribution		
			G1	S	G_2/M
– PHA	72	0	92.2 ± 0.4	1.3 ± 0.5	6.6 ± 0.1
– PHA	72	1	91.5 ± 0.4	1.1 ± 0.4	7.4 ± 0.0
– PHA	72	3	91.1 ± 0.8	1.4 ± 0.6	7.6 ± 0.1
– PHA	72	5	92.8 ± 0.6	1.2 ± 0.5	6.1 ± 0.1
+ PHA	72	0	63.5 ± 4.1	28.1 ± 1.8	8.4 ± 2.3
+ PHA	72	1	56.4 ± 2.3	33.7 ± 2.2	9.9 ± 0.1
+ PHA	72	3	64.6 ± 1.6	26.8 ± 1.2	8.6 ± 0.4
+ PHA	72	5	64.7 ± 1.5	26.9 ± 1.3	8.5 ± 0.1

FIG. 3. Cell cycle effects of different concentrations of 70% ethanol and water extracts of I'm-Yunity™ (Integrated Chinese Medicine Holdings Ltd., Kowloon, Hong Kong) in nonstimulated and phytohemagglutinin (PHA)- stimulated (PBL). The upper panels show the DNA content frequency histograms of nonstimulated (−PHA) and mitogen (+PHA) stimulated lymphocytes grown for 72 hours with different concentrations of water extracts of I'm-Yunity. MultiCycle software (Phoenix Flow Systems, San Diego, CA) was used to deconvolute the cellular DNA content frequency histograms to obtain the percentage of cells in the respective phases (G_1, S and G_2/M) of the cell cycle to obtain the data as shown in Table 1. Apoptotic cells were identified on the DNA histograms as "sub-G_1" cells.

DISCUSSION

The global cancer incidence is soaring as a result of rapidly aging populations in most countries. By the year 2020, there will be 20 million new patients with cancer each year. A large percentage of them will live in countries that lack the resources for cancer control. Unquestionably, the ability to prevent the development of cancer and efforts at early detection of

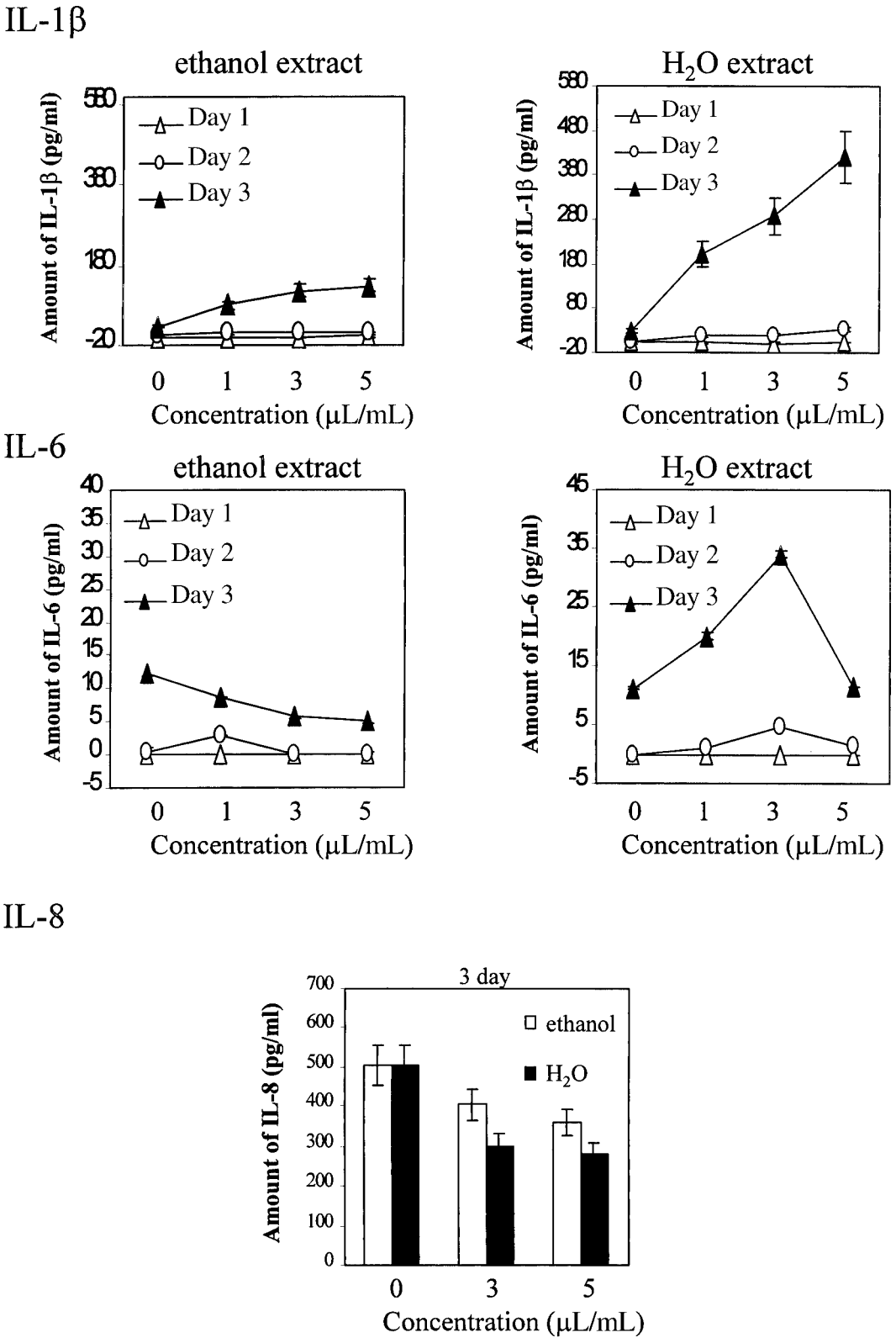


FIG. 4. Effect of different concentrations of I'm-Yunity™ (Integrated Chinese Medicine Holdings Ltd., Kowloon, Hong Kong) on expression of interleukins. Measurements of interleukin (IL)-1*β*, IL-6, and IL-8 using media of HL-60 cells incubated with various concentrations of ethanol or water extracts of I'm-Yunity were as described in Materials and Methods.

TABLE 2. EFFECTS OF ETHANOL AND AQUEOUS EXTRACTS OF I'M-YUNITY™ [a] ON RELATIVE CHANGES OF IL-1β/IL-8 AND IL-6/IL-8

Treatment	*I'm-Yunity*™ [a] (μL/mL)	*Relative changes in* IL-1β/IL-8	*Relative changes in* IL-6/IL-8
Control	3	1.00	1.00
Ethanol (70%)	3	5.11	0.58
H_2O	3	17.14	4.71

[a]Integrated Chinese Medicine Holdings Ltd., Kowloon, Hong Kong.

IL, interleukin; H_2O, water.

curable neoplastic lesions are likely to have greater impact on the morbidity and mortality associated with the diagnosis of cancer, and these strides, coupled with dramatic technological changes that will continue in surgery, radiotherapy, and chemotherapy will lead to increased cure rates. These anticipated advances, however, come at a price usually beyond the means of most cancer patients. Because the optimal organization of prevention and detection programs as well as treatment services are projected to be universal problems in all economic environments, it is imperative that due considerations be given to development of affordable alternative/complementary preventive and treatment strategies that could considerably reduce the global disease burden at manageable costs.

Historically, in certain cultures botanicals, plant-derived supplements, and extracts of mushrooms have been considered as important remedies for maintaining health, enhancing overall immune status, and for prevention and treatment of chronic diseases (Spencer, 1999). In recent years, these natural products are also receiving increasing interest and broader use in the United States for a variety of conditions (Go et al., 2001). A requisite in the proper evaluation of biologic efficacy for these dietary supplements is a better understanding of their mechanism, in both normal and malignant cells.

Numerous studies have shown that mushroom extracts possess a plethora of biologic activities (Borchers et al., 1999; Kidd, 2000; Wasser and Weis, 1999; Ng, 1998). The mushroom *Coriolus versicolor*, known in China as Yun-Zhi, has been used in herbal medicine for more than 2000 years (Lee et al., 1984). Previous studies have focused on PSP, a bioactive polysaccharide-peptide extracted from deep-layer mycelia of Cov-1, which is a patented strain of *Coriolus versicolor* identified from a screen of more than 80 strains (Jong and Yang, 1999; Kidd, 2000). PSP has demonstrated antiviral, antitumor and immune enhancing activities (Kidd, 2000; Yang, 1997). To our knowledge, its effects in leukemia cells have not been investigated nor has a comparison been made of its activities in normal lymphocytes.

In the present investigation, we studied the cellular effects of water and 70% ethanol extracts prepared from the PSP, I'm-Yunity, in HL-60 leukemia cells. This cell line has been used extensively to probe into the basis as well as possible treatment modalities of acute leukemia (DiPetrantonio et al., 1996, 1998, 2000). Both extracts had a potent cytostatic effect on the growth of HL-60 cells and led to accumulation of cells in the G_1 phase of the cell cycle. Because the accumulation of cells in G_1 was also observed at the extract concentrations that did not induce detectable apoptosis, most likely this effect reflected cell arrest in G_1 rather than a selective cell death in S and/or G_2/M (Fig. 2 and Table 1). The water extract, however, not only was more effective in blocking G_1 but at the higher concentrations (7.5 and 10 μL/mL) also led to accumulation of HL-60 cells in G_2/M. At these high extract concentrations significant number of cells were undergoing apoptosis as well. It is possible, therefore, that either high concentrations of the water extract led to cell arrest in G_2/M or preferentially induced apoptosis of S-phase cells, or both effects occurred simultaneously. These data indicate that the most potent bioactive ingredients in I'm-Yunity are water soluble. It is likely that they elicit multiple biologic responses, as evidenced by the accumulation of HL-60 cells in G_1 at low concentration of the extract and an increase in their proportion at G_2/M at high extract concentration.

The mechanism responsible for high sensitivity of HL-60 cells compared to normal lymphocytes, which showed significant resistance to the extracts, is unclear. The difference does not appear to be related to the proliferative sta-

tus of the cells because both HL-60 cells and PHA-stimulated lymphocytes were progressing through the cell cycle, and the rate of the cell cycle progression is similar for both cell types. Because disregulation of the retinoblastoma pathway, the pathway that controls the cell cycle progression through G_1 (the passage of restriction point; R), appears to be the common cellular defect of most cancers including leukemia (Bartek et al., 1997), the increased sensitivity of HL-60 cells to I'm-Yunity, compared to lymphocytes, may be associated with this defect.

We also assessed the ability of extracts to modulate immune functions by measuring changes in the secreted IL-1β, IL-6, and IL-8. IL-1β is produced by a large number of cell types including blood cells (Allen et al., 1992). In addition to its well-established role in initiation of inflammation, IL-1β is also associated with other functions such as bone formation and remodeling, insulin secretion, appetite regulation, fever induction, neuronal phenotype development, and insulin-like growth factor/growth hormone physiology (Allen et al., 1992; Watkins et al., 1999). IL-6 is a multifunctional protein produced by lymphoid and nonlymphoid cells, and by normal and transformed cells. Similar to IL-1β, IL-6 reportedly has multiple functions that include stimulation of differentiation and antibody secretion in B cells, promotion of differentiation of cytotoxic T cells, induction of acute phase proteins by hepatocytes, stimulation of colony formation on hematopoietic stem cells (Taga and Kishimoto 1997; Van Snick and Nordan, 1990), and enhancement of the retinoic acid-induced differentiation of human promyelocytic leukemia cells (Xie et al., 2000). The various activities of IL-1β and IL-6 described above suggest that these factors have a major role in the mediation of the inflammatory and immune responses. Our studies show that both extracts significantly increased the secretion of these two cytokines in HL-60 cells. Interestingly, IL-8, an inflammatory cytokine that has been associated with angiogenesis and the expression of which appears to be correlated with tumorigenesis (Inoue et al., 2000; Kitadai et al., 1999), is significantly reduced when HL-60 cells were incubated with either extracts (Fig. 4 and Table 2).

The full implications of these results remain to be elucidated. Given that the cell-cycle–restrictive and apoptosis-inducing properties of I'm-Yunity are complemented by their immunomodulatory attributes, it seems possible that this dietary supplement could offer beneficial effects in individuals diagnosed with leukemia, both by selectively curtailing cancer cell growth and by enhancing immune functions. Clearly, the observed cell cycle effects (G_1 and G_2/M arrest) and induction of apoptosis of HL-60 cells are characteristic of many cancer-preventive and chemotherapeutic agents. It is unknown, however, whether the concentration of the active components of this form of PSP from *Coriolus versicolor* are achievable *in vivo*, in tissues of the patients taking this herbal supplement.

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